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The effects of temperature on the dark-adapted spectral sensitivity function of the adult zebrafish

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Abstract

In goldfish and other cold-blooded vertebrates, temperature can influence the rhodopsin/ porphyropsin contributions to the rod photoreceptors. This study examined the effects of temperature on the spectral sensitivity function of the dark-adapted zebrafish. Zebrafish were housed in either a warm (28–30°C) or cold (22–25°C) tank prior to testing. Fish were dark-adapted for at least 1 h and electroretinogram (ERG) responses to 200 ms stimuli of various wavelengths and irradiances were obtained. Results show that water temperature affected the spectral sensitivity function of the ERG b-wave. Subjects housed in the warm temperatures had a spectral sensitivity consistent with the rhodopsin absorption curve; however, fish housed in the colder temperatures had a spectral sensitivity function that was the result of a rhodopsin/porphyropsin mixture. In addition, ultraviolet cones (λ_{max}: 362 nm) contributed to the dark-adapted spectral sensitivity function under both temperature conditions. Consistent with the results from other fish, the dark-adapted visual system of the zebrafish can be influenced by water temperature. The results of this study demonstrate the necessity of maintaining a stable environment when examining the contributions of the photoreceptors to the visual response.

Keywords: *Danio rerio*; Dark-adapted spectral sensitivity; Electroretinogram; Temperature

1. Introduction

It has been shown that certain environmental factors coincide with changes in the type of photopigment contained in the rods of lower vertebrates. Seasonal effects have been associated with whether a species has a dark-adapted visual system dominated by rhodopsin, porphyropsin or a visual system with both pigments. A general finding across a number of different fish species, including the golden shiner (*Notemigonius aureatus*) (Allen & McFarland, 1973) and the rudd (*Scardinius erythrophthalmus*) (Knowles & Dartnall, 1977) is that porphyropsin dominates the rod visual system during the winter months, while rhodopsin is typically found during the summer months.

It is not certain whether the changes that occur with dual pigment species under normal conditions are caused by changes in the lighting environment such as the spectral characteristics of the light, day length, or some other factor associated with seasonal changes such as temperature. Interestingly, the shift from rhodopsin to porphyropsin has not always been consistent with changes in the spectral characteristics of the light environment. One would expect a shift from rhodopsin (λ_{max} of 500 nm) to porphyropsin (λ_{max} of 523 nm) when the light environment changes towards longer wavelengths, and vice versa. However, this is not always the case (Bowmaker, 1995).

A change in water temperature seems to be a coincidental factor of the seasonal changes that occur in the natural environment of certain species. However, it appears that temperature also may affect the rod visual system of fish. Allen and McFarland (1973) examined rod photopigment composition of the golden shiner at various times of the year and found that during the winter months, when the temperature was 2–4°C, there was a large percentage of porphyropsin which dropped during the summer months when the temperature was 16–18°C.

Even fish that typically are not considered to be a dual pigment species appear to be able to modify their

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rhodopsin/porphyropsin ratio. Temperature effects on rod photopigment composition have been studied in goldfish. Under normal conditions, the goldfish is reported to possess solely porphyropsin (Schwanzara, 1967). However, Tsin and Beatty (1979) found that increasing the water temperature produced a shift in the visual pigment composition from porphyropsin to rhodopsin. This raises the issue of whether all fish, both migratory and non-migratory, have the ability to alter their photopigment composition under the appropriate conditions.

The zebrafish (*Danio rerio*), like the goldfish, is a non-migratory fish. However, like many tropical fish, the zebrafish has rhodopsin in its rod photoreceptors (Schwanzara, 1967). The main purpose of this work was to examine whether water temperature affects the dark-adapted spectral sensitivity function of the zebrafish, with lighting conditions held constant. Thus, this study will determine whether temperature alone can be responsible for a shift in the rod visual pigment in a non-migratory tropical fish.

Additionally, this work will examine whether there are cone contributions to the dark-adapted spectral sensitivity function of the zebrafish. Dark-adapted spectral sensitivity in some fish species, including goldfish (Nussdorf & Powers, 1988) and giant Danio (*Danio aequipinnatus*) (van Roessel, Palacios & Goldsmith, 1997), appears broader (more sensitive) at the longer wavelengths than one would expect from their reported rod photopigment spectra. In both the dark-adapted goldfish and giant Danio, it has been suggested that the increased sensitivity found at the longer wavelengths is due to the contribution of the long-wavelength cones or L-cones (Powers & Easter, 1978; Nussdorf & Powers, 1988). Given that research has shown that the zebrafish and the goldfish visual systems are somewhat similar, it is feasible that the two may have similar properties under dark-adapted conditions. In addition, since the zebrafish and the giant *Danio* are closely related, one would expect their dark-adapted visual systems to be similar. Therefore, one would predict that there would be cone contributions to the zebrafish dark-adapted spectral sensitivity function.

2. Methods

2.1. Participants

Adult male and female zebrafish (*D. rerio*) ranging in size from 4 to 5 cm in length were used. The fish were obtained from Scientific Hatcheries (Huntington Beach, CA) and a local pet store. They were maintained on a 14 h light/10 h dark cycle for at least 2 weeks prior to testing and fed daily with tropical fish food flakes. Participants were housed in either a warm tank which had a temperature of 28–30°C or a cold tank which had a temperature of 22–25°C. The warm temperature condition represents standard zebrafish rearing conditions (Westerfield, 1994). Since temperatures lower than 22°C have been shown to have detrimental effects on zebrafish development (Schirone & Gross, 1953), we restricted our lowest temperature to 22°C. The fish were allowed to adjust to the tank temperature for at least 3 days prior to testing.

2.2. Apparatus

2.2.1. Electrophysiological apparatus

Electroretinograms (ERGs) were recorded with a 36 gauge chlorided silver electrode positioned in the vitreal chamber. Another 36 gauge chlorided silver electrode, placed in the nostril, was used as a reference. Electrical signals were passed through a differential amplifier (WPI, Sarasota, FL, DAM-50) with a band-pass of 0.1–100 Hz and sent simultaneously to an oscilloscope and the laboratory computer. The sampling rate of the computer's data acquisition board was 4 ms.

2.2.2. Optical system

The optical system's light source was a 150 W xenon arc lamp. The light passed through interference filters with a half-bandwidth of 10 nm to control stimulus wavelength. A series of quartz neutral density filters were used to control stimulus irradiance and could attenuate the light over a 9 log unit range. The light was focused onto one end of a liquid light guide; the other end was placed in front of the subject's eye. Light measurements were converted to quanta s^{-1} cm^{-2} from the values provided by a radiometer (International Light, Newburyport, MA, IL1400) sensitive to ultraviolet and visible wavelengths.

2.3. Procedures

Each subject was anesthetized by immersion in a 0.04% solution of tricaine methanesulfonate (MS-222) and then paralyzed by an intramuscular injection of 20 μg of gallamine triethiodide. A hole was made in the sclera of the eye using a 26 gauge needle to allow the entry of the ERG electrode. The subject was then placed inside a stereotaxic device and into a plexiglas tank which was located in a light tight Faraday cage. Subjects were artificially respired by a water pump which circulated an aerated solution of water and 0.01% MS-222 over the gills throughout the experiment. Water entered the plexiglas tank via a tube which was placed in the subject's mouth. An exit hole was made in the back of the plexiglas tank for drainage. Once the animal was positioned in the recording chamber, the subject was dark-adapted for at least 1 h.

Monochromatic light of varying wavelengths and irradiances were presented. A total of 18 wavelengths from 320 to 660 nm in 20 nm steps were used. Presentation of the wavelengths was staggered in 40 nm steps for the first series starting at either 320 or 660 nm, and then on the second series, the remaining wavelengths were filled in such that 20 nm steps existed in the data. Stimulus duration was 200 ms; to maintain the dark-adapted state, a 1 min delay was used between stimulus trials. For each wavelength, trials began at an irradiance in which a b-wave response could not be measured reliably. The irradiance was then increased in 0.5 log unit steps until a criterion b-wave response of + 50 μV was obtained.

3. Results

Spectral sensitivity functions were obtained from the b-wave component of the ERG waveform averaged across three stimulus presentations. Fig. 1 shows the ERG response of a dark-adapted adult zebrafish to a 500 nm stimulus of various irradiances. Each waveform was initially filtered for 60 Hz noise prior to response averaging. The horizontal line illustrates the onset and termination of the stimulus; the values associated with each response represent the log stimulus attenuation where 0.0 corresponds to a log irradiance of 15.26 log quanta s^{-1} cm^{-2}. As Fig. 1 shows, the ERG b-wave amplitude increases with stimulus irradiance. Sensitivity to each stimulus wavelength was defined as the reciprocal of the log stimulus irradiance (quanta s^{-1} cm^{-2}) which yielded a + 50 μV b-wave response amplitude. A 50 μV criterion response was chosen because this response always was above noise and below response saturation. Thus, this value was reliably within the linear portion of the function (Hughes, Saszik, Bilotta, DeMarco, & Patterson, 1998), and any response criterion within this portion of the response function is arbitrary on a relative scale and does not alter the shape of the spectral sensitivity function. Finally, a response criterion of 50 μV has been used to derive dark-adapted spectral sensitivity functions from ERG b-wave responses in goldfish (Nussdorf & Powers, 1988; DeMarco & Powers, 1989).

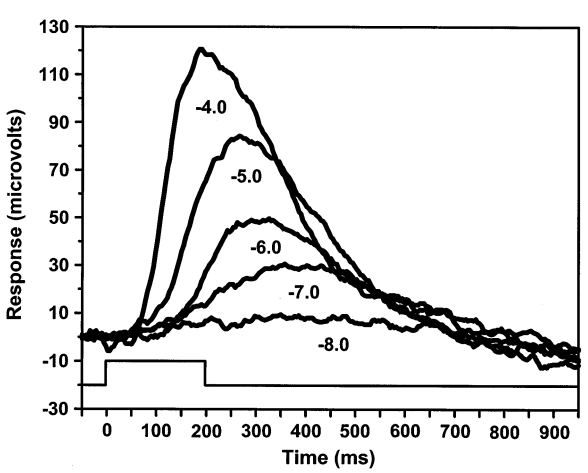

Fig. 1. Dark-adapted zebrafish ERG responses to a 500 nm stimulus at various irradiances. The horizontal line illustrates the onset and termination of the stimulus. Negative values below each waveform represent the log stimulus attenuation where 0.0 corresponds to a log irradiance of 15.26 log quanta s^{-1} cm^{-2}.

To determine the photoreceptor and/or photopigment contribution to the spectral sensitivity function, a linear-additive model used successfully with data from both the goldfish (DeMarco & Powers, 1991) and the trout (Coughlin & Hawryshyn, 1994) was used. The linear-additive model allows one to determine input weights for the type of photoreceptor and/or photopigment and has the following form:

$$S_\lambda = \sum_{i=1}^{w} (K_x \times A_x) \quad (1)$$

where S_λ, is the spectral sensitivity at wavelength λ, A_x the absorptance of photoreceptor/photopigment x at wavelength λ, K_x the weight of photoreceptor/photopigment x, and w the number of photoreceptor/photopigments contributing to the model.

The cone spectra were obtained from intracellular recordings from the giant danio (Palacios, Goldsmith & Bernard, 1996) placed on a normalized frequency axis using the λ_{max} ($\lambda_{max} = 362$, 415, 480, and 570 nm; U-, S-, M-, and L-cones, respectively) from zebrafish microspectrophotometric data (Robinson, Schmitt, Harosi, Reece & Dowling, 1993). These cone spectra fit the photopic spectral sensitivity data obtained from zebrafish ERG b-wave responses (Hughes, et al., 1998). The rhodopsin absorption curve was obtained from Dartnall (1953) and the porphyropsin absorption curve was obtained from Bridges (1967).

Sensitivity values were converted to proportions and then normalized to their maximum value (DeMarco & Powers, 1991). A simplex algorithm was used to determine the photoreceptor/photopigment weights which yielded the best-fit model to the data. To find the best-fit model with the appropriate number of photoreceptor/photopigment contributions (i.e., w in Eq. (1)), several approaches could be taken. One approach is to include all of the possible inputs (i.e. $w = 6$; all four cone types with the two rod photopigments) and determine if any photoreceptor types mathematically fall out of the model. However, with any least-squares model, more variables always will account for more variance than fewer variables. Thus, more variables can produce a statistical artifact that is unrelated to the actual inputs contributing to the response. In addition, there are theoretical reasons not to include cone contributions to the dark-adapted visual system. For these reasons, we

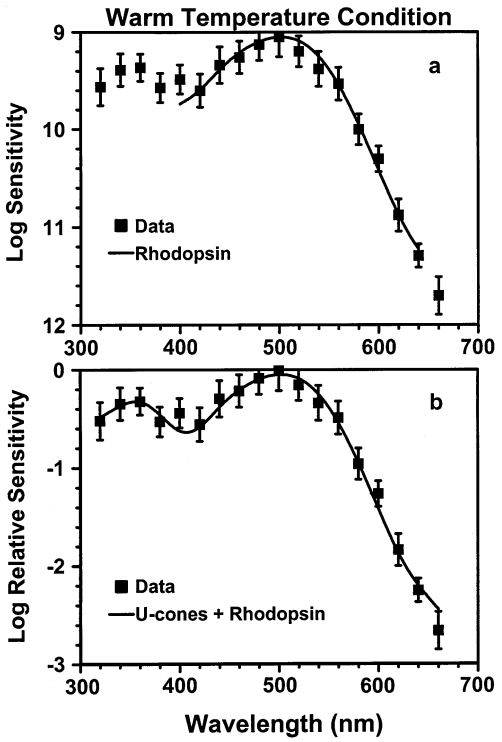

Fig. 2. (a) Averaged dark-adapted spectral sensitivity function from ten subjects (squares) obtained under the warm temperature condition. Log sensitivity (log quanta s^{-1} cm^{-2}) is defined as the reciprocal of the log stimulus irradiance that produced a 50 μV b-wave response. Rhodopsin nomogram (line) was fit by eye to the data. Error bars equal ±1 S.E.M. (b) Averaged dark-adapted spectral sensitivity function (squares) plotted with the results of the linear-additive model (line). Other details as in Fig. 2a.

adopted a conservative approach to fit the spectral sensitivity data.

Preliminary analysis involved fitting the cone and rod spectra (A_x) by eye to the spectral sensitivity data under each temperature condition. Following this analysis, rod and cone spectra were added one by one and the variance accounted for by the model (R^2) was examined. Once the variance accounted for reached a plateau, the modeling was complete. This preliminary analysis revealed that the S-, M-, and L-cones did not contribute to the dark-adapted spectral sensitivity function. When these cone types (including the L-cones) were placed into the model, the analysis which produced the best-fitting final model set their weights to near zero. Incorporating these cone types into the model never produced a better model than when they were not included. Therefore in the final models, these cones types were not included, leaving the U-cones, rhodopsin, and porphyropsin.

3.1. Warm temperature condition

Fig. 2a shows the mean spectral sensitivity function from ten subjects in the warm temperature condition. The data (squares) have been fit by eye to the rhodopsin absorption curve (line). The rhodopsin absorption curve fits the dark-adapted spectral sensitivity data well from 400 to 660 nm under the warm temperature condition. To support the fit by eye, the data were modeled using the linear-additive model. Fig. 2b shows the data (squares) plotted with a model which included U-cones and rhodopsin; the model is represented by the solid line on the graph. Like the fit by eye, the mean data from the subjects in the warm temperature condition fit the rhodopsin spectra from 400 to 660 nm. In addition, to account for the data from 320 to 380 nm, a U-cone input was required. Finally, the amount of contribution from porphyropsin for the subjects in the warm temperature condition was negligible (see Table 1). In fact, the variance accounted for by the model which included the small contributions of porphyropsin was identical to the variance accounted for when the porphyropsin input was excluded from the model.

3.2. Cold temperature condition

Fig. 3a shows the mean spectral sensitivity function of ten subjects in the cold temperature condition. As was done with the data from the warm temperature condition, the data (circles) were first fit by eye to the rhodopsin absorption curve. Using this curve (solid line) it was found that the data points at the higher wavelengths (i.e. 540–660 nm) were consistently above the rhodopsin spectra. For this reason the data also were fit by eye with the porphyropsin (dashed line) absorption curve. The data points at the middle wavelengths seemed to fit well, as with the rhodopsin absorption curve, but at the longer wavelengths the data points were consistently below the porphyropsin spectra. It is apparent from this figure that there is possibly some combination of the two rod photopigments.

The cold temperature data was modeled using the same procedure as the data in the warm temperature condition. The results of this analysis are seen in Fig. 3b. The solid line represents the model which includes the U-cones, rhodopsin, and porphyropsin, while the circles are the data. The model does fit the data well, and the model weights further show support for a combination of rhodopsin and porphyropsin (see Table 1). Instead of the full weight being given to the rhodopsin contribution from 400 to 660 nm, as was the case with the data from the warm temperature condi-

Table 1
Linear-additive model weights

Temperature	U-cones	Rhodopsin	Porphyropsin	R^2
Warm	0.22	0.96	−0.05	0.96
Cold	0.45	0.79	0.36	0.86

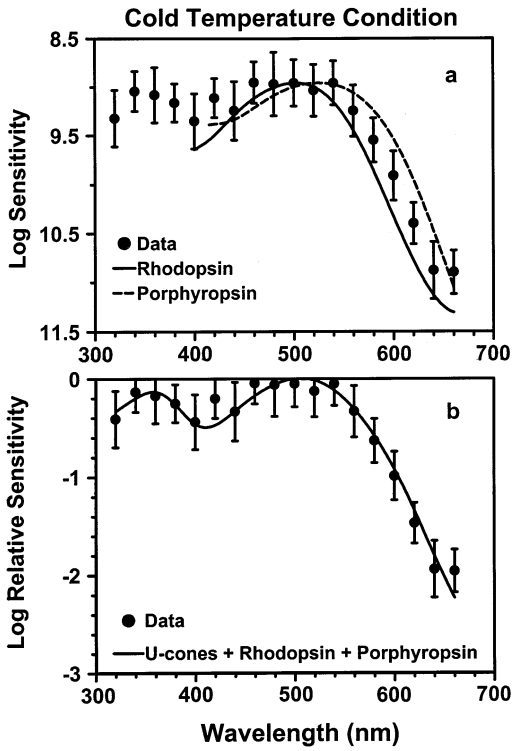

Fig. 3. (a) Averaged dark-adapted spectral sensitivity function from ten subjects (circles) obtained under the cold temperature condition. Log sensitivity (log quanta s^{-1} cm^{-2}) is defined as the reciprocal of the log stimulus irradiance that produced a 50 μV b-wave response. Rhodopsin (solid line) and porphyropsin (dashed line) were fit by eye to the data. Error bars equal ± 1 S.E.M. (b) Averaged dark-adapted spectral sensitivity function (circles) plotted with the results of the linear-additive model (line). Other details as in Fig. 3a.

tion, porphyropsin was shown to have contributed to the spectral sensitivity function under the cold temperature condition.

Table 1 shows the values of the best-fit linear-additive model under each temperature condition. The weights of the two rod photopigments and the U-cones are shown for each temperature condition, as well as the resulting R^2 value for each model. Comparing the values of the two rod photopigments under the warm temperature condition (model weights of 0.96 and − 0.05 for rhodopsin and porphyropsin, respectively), it is evident that the data from 400 to 660 nm from the warm temperature condition are consistent with the rhodopsin spectra. However, under the cold temperature condition, instead of having a system dominated by rhodopsin, there is a combination of rhodopsin and porphyropsin (model weights of 0.79 and 0.36 for rhodopsin and porphyropsin, respectively).

It is also interesting to note that under both temperature conditions, the U-cones make a substantial contribution to the response to the ultraviolet wavelengths (i.e. 320–380 nm). The models shown in Table 1 yielded the highest R^2 values (i.e. accounted for the most variance) of all the other models tested, except for the full model (i.e. $w = 6$ in Eq. (1)). The R^2 for the full model under the warm and cold temperature conditions were 0.97 and 0.91, respectively.

Fig. 4 shows a bar graph with the relative proportion of both rod photopigment contributions under the two temperature conditions. The relative porphyropsin weight was determined by normalizing the porphyropsin weight obtained from the model with respect to the rhodopsin weight. One can see that there is a large contribution of the porphyropsin photopigment under the cold temperature condition, but under the warm temperature condition, the contribution from porphyropsin is essentially nonexistent.

4. Discussion

There were two main outcomes of the present study. First, the results clearly show that the dark-adapted spectral sensitivity function of the zebrafish is affected by water temperature. Subjects housed in warm water displayed a dark-adapted spectral sensitivity function that corresponded to the rhodopsin photopigment spectra. However, fish housed in colder water displayed a spectral sensitivity function that appeared to reflect the contributions of both rhodopsin and porphyropsin pigments. In addition, it was shown that the dark-adapted spectral sensitivity function receives U-cone contributions. This is apparent by the increased sensitivity of the zebrafish to ultraviolet wavelengths.

The effects of temperature on the spectral sensitivity function derived from the ERG b-wave are consistent with temperature effects found for goldfish photopig-

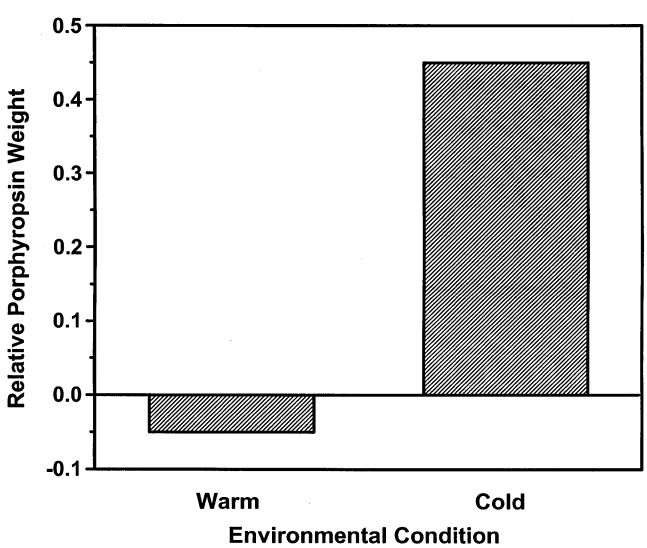

Fig. 4. Relative contributions of rhodopsin and porphyropsin to the spectral sensitivity data under the two temperature conditions. The relative porphyropsin weight was derived by normalizing the weight obtained from the linear-additive model relative to the obtained rhodopsin weight. See text for details.

ment composition. Under the temperature conditions used in this study, there was a shift from a system dominated by rhodopsin under warm temperature conditions to one that showed a combination of rhodopsin and porphyropsin contributions under cold temperature conditions. Tsin and Beatty (1979) showed in goldfish that as water temperature decreases, there is a shift in the rod photopigment composition from rhodopsin to porphyropsin. The temperatures used in the present study were similar to those used by Tsin and Beatty (1979). According to their results, housing goldfish in our cold water condition (22–25°C), would yield a mixture of rhodopsin and porphyropsin pigments. However, if the temperature in the present study had been lower as was the case with the goldfish (e.g. 10°C), there may have been a complete shift from rhodopsin to porphyropsin in zebrafish.

Although seasonal changes in the photic environment, such as daylength, the intensity of the light, as well as the spectral quality of the light, are important, the results from the present study show that temperature also may play a role in rod photopigment composition. The shift from rhodopsin to a mixture of the two pigments found in the present study is consistent with the results of Allen and McFarland (1973). During the winter months, when the temperatures were lower, they found a higher proportion of porphyropsin in the golden shiner, while during the warmer summer months, there was a higher proportion of rhodopsin photopigment. These results as well as those of the present study illustrate the importance of controlling other environmental factors, such as temperature, as well as changes in the light environment. It may be possible that some of the discrepancies found across the various studies attributed to species differences may be due to extraneous variables such as temperature.

Recently, van Roessel, et al. (1998) showed that the dark-adapted spectral sensitivity function derived from the ERG b-wave response of giant Danio is broader than one would predict from the rhodopsin photopigment. They have suggested that the L-cones contribute to the dark-adapted spectral sensitivity function which would explain the increased sensitivity that is apparent at the longer wavelengths. Our attempts at modeling found no contributions from L-cones (as well as the S- and M-cones). However, given that the two species are similar, one would expect similar results. The present study has shown that the zebrafish dark-adapted spectral sensitivity function can be best fit by either the rhodopsin or a mixture of rhodopsin/porphyropsin depending on the temperature that the fish were housed. It is possible that the giant Danio also may be affected by water temperature. Perhaps the increased sensitivity found in the giant Danio at the longer wavelengths may be the result of contributions of porphyropsin photopigment to the dark-adapted function (the giant Danio data was modeled with the rhodopsin photopigment). It should be noted that the giant Danio was housed at a water temperature of 25°C, a temperature that fell within the cold temperature conditions used in the present study.

These results, in light of other similar findings, suggest that the effects of the environment on the composition of the visual pigments may not be species dependent. The zebrafish shows the same shift as other fish species in the composition of the rod photopigment under similar temperature conditions. This shift occurred even though these environmental changes may not occur in the animal's natural environment. Zebrafish are warm-water fish and most likely do not experience such dramatic changes in temperature in their normal environment. This leads one to believe that this potential might exist in other fish species (and perhaps other cold-blooded species) that have been thought to have a visual system dominated by only one type of photopigment. Having shown this phenomenon in both the zebrafish and goldfish, neither of which experiences a dramatic seasonal change in its environment, supports this notion. In addition, the present study showed that the zebrafish adjusted quickly to the change in water temperature. Subjects moved from warm temperature tanks to cold temperature tanks displayed the porphyropsin/rhodopsin mixture within three days. Although this transition may seem rather abrupt, Allen (1971) reported a change in the rod pigment composition of the red shiner within 7 days. It should be mentioned that there was no difference in the rod photopigment mixture as a function of duration. Spectral sensitivities were similar across subjects regardless of how long they were in the particular temperature condition as long as it was longer than 3 days; we did not test earlier than 3 days.

The other major finding of the present study is the apparent contributions of the U-cones to the dark-adapted spectral sensitivity function. The rod photopigments (either rhodopsin in the warm temperature condition or a rhodopsin/porphyropsin mixture in the cold temperature condition) appear to fit the spectral sensitivity functions from 400 to 660 nm. However, to account for the data from 320 to 380 nm, a U-cone component was needed. The spectral sensitivity functions of both groups display a peak in the ultraviolet region of the spectrum. This peak is at a much lower wavelength than the peak of either rhodopsin or porphyropsin. At first this peak might be attributed to the β-band of either of the rod photopigments, but that probably is not the case. First, if the peak at 360 nm were the β-band, its sensitivity value probably would be lower than the peak of the rod α-band. In addition, if this peak were the β-band of the rod photopigment, one would expect to find this peak at a lower wavelength under the warm temperature condition, which is

dominated by rhodopsin, compared to that under the cold temperature condition, which consists of a rhodopsin/porphyropsin photopigment mixture. This was not the case, since the secondary peaks under both conditions are found at about 360 nm. Finally, this peak matches that of the U-cone λ_{max} of 362 nm (Robinson, et al., 1993).

It is interesting to note that even though there was shift in the rod photopigment with temperature, there did not appear to be a shift in the cone photopigment composition. This is supported by the fact that the peak sensitivity found at the ultraviolet wavelengths did not shift across the two temperature conditions. In addition, we have found that under photopic conditions, the spectral sensitivity function did not appear to change with changes in water temperature (unpublished observations).

It is apparent from the results, that the U-cones do contribute to the dark-adapted spectral sensitivity function of the adult zebrafish. The purpose of this contribution is not clear. It is possible that the purpose of U-cone contribution to the dark-adapted function is to expand the range of wavelengths to which the zebrafish is sensitive under dark-adapted conditions. Under daylight conditions there is an abundance of ultraviolet light present in their environment (Jerlov, 1968, cited in Nicol (1989)), but under dark conditions it is not clear whether the same is true. In order to address this issue, more details about the zebrafish natural environment under dark-adapted conditions would be needed.

The present study supports the notion that the goldfish and zebrafish do have similar properties under dark-adapted conditions in that there are cone contributions to the spectral sensitivity function. For the goldfish, however it is the L-cones that are contributing to the dark-adapted spectral sensitivity function (Nussdorf & Powers, 1988). In goldfish, the L-cones appear to be an important cone type. There are more L-cones (45%) than the other remaining cone types (Marc & Sperling, 1976). Also, their input to ganglion cells is prominent regardless of ganglion cell type (Mackintosh, Bilotta & Abramov, 1987). Thus, given that the L-cones are such a dominant cone type in the goldfish visual system, it is not surprising that if there were cone contributions to the goldfish dark-adapted visual system, that it would be the L-cones.

On the other hand, the U-cones appear to play an important role in the zebrafish visual system. They comprise 25% of the cones on the retina (Robinson et al., 1993) and contribute to the photopic spectral sensitivity function in the adult zebrafish (Hughes et al., 1998). In addition, recent studies have shown that they may be the first functional cone type in the developing zebrafish retina. Miller, Bilotta & Givin (1997) have shown that the spectral sensitivity derived from the ERG b-wave of very young zebrafish (6–8 days postfertilization), when rods are not present, possesses primarily U-cone contributions; the contributions of the L-cones at this age are minimal. Given these results, it is not surprising that if there were cone contributions to zebrafish dark-adapted vision that it would be the U-cones. The presence of the U-cones does not mean that the zebrafish have color vision under dark-adapted conditions, but that their visible spectrum is much larger by including both rod and U-cone input.

In summary, the results of this study have shown that the zebrafish dark-adapted spectral sensitivity function is affected by the temperature at which the subjects are housed. Temperature appears to affect the type of rod photopigment contributing to the ERG b-wave response. As water temperature decreases, rhodopsin contributions decrease and porphyropsin contributions increase. In addition, the ultraviolet cones contribute to the dark-adapted spectral sensitivity function in zebrafish, demonstrating that under optimal dark-adapted conditions cone systems can be as sensitive as rods. These results support the importance of maintaining a stable environment when studying photoreceptor contributions to vision. Also, these results suggest that caution should be used when classifying species as single versus dual pigment systems; the distinction may not be as clear-cut as originally believed.

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